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Citation for published version:
Fray, MD, Pickard, AR, Harrison, M & Cheeseman, MT 2008, 'Upgrading mouse health and welfare: direct benefits of a large-scale rederivation programme', *Laboratory Animals*, vol. 42, no. 2, pp. 127-139. https://doi.org/10.1258/la.2007.007005

Digital Object Identifier (DOI):
10.1258/la.2007.007005

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Laboratory Animals

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Lab Anim 2008 42: 127
DOI: 10.1258/la.2007.007005

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Upgrading mouse health and welfare: direct benefits of a large-scale rederivation programme

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Summary

We report the outcome of a 30-month programme to rederive 310 specific pathogen-free mouse strains to populate a new individually ventilated cage barrier facility at the Mary Lyon Centre (MLC), Medical Research Council (MRC) Harwell. The mice were rederived in a self-contained quarantine suite and embryo-recipient females were health-screened to assess microbiological status, before moving their offspring into the new facility. The MLC currently houses approximately 49,000 mice in about 9750 cages and we have 30 months of follow-up health screen data. Embryo rederivation and hysterectomy have high safety margins; however, the precaution of performing the programme in isolators facilitated the containment and decontamination of two mouse hepatitis virus (MHV) infection outbreaks. Rederivation of the colony has eliminated endemic MHV, mouse adenovirus type 2 (MAV-2), Theiler's murine encephalomyelitis virus, pinworms, intestinal protozoa, *Pasteurella pneumotropica, Helicobacter* spp. and mites. The improvements in microbiological status have had notable benefits for mouse health and welfare and the science at MRC Harwell. Previously important clinical entities such as sudden death associated with lactation ileus in C3H/HeH mice, early weight loss associated with inflammatory bowel disease in B6-TgN(HDexon1)61Gpb and B6-TgN(HD82Gln)81Dbo (Huntington) mice and early weight loss in male mice mutagenized with *N*-ethyl-*N*-nitrosourea have been markedly reduced or eliminated.

Keywords Embryo transfer; *in vitro* fertilization (IVF); specific pathogen free (SPF); health; welfare

Upgrading mouse house facilities presents a unique opportunity to improve the microbiological status of stocks and thereby improve the health and welfare of mice. In addition to the effort to upgrade mouse facilities, Europe's scientific community is making a concerted effort to standardize the health-screening programmes (e.g. Federation of European Laboratory Animal Science Associations [FELASA] recommendations; Nicklas *et al.* 2002) to achieve better standards of health and thereby improve the characterization of mouse models of mammalian physiology and human disease. There is also recognition of the need to generate reproducible results for a series of standardized mouse models in multicentre studies, e.g. EUMORPHIA, EUROHEAR, EUMODIC and other integrated research programmes.

This paper discusses the strategy, costs and benefits of a large-scale, 30-month programme to rederive 310 mouse strains into the Mary Lyon Centre (MLC), a new individually ventilated cage (IVC) barrier facility with a maximum cage capacity of 13,000. This specific pathogen-free (SPF) facility currently houses approximately 49,000 mice in about 9750 cages and the two pre-existing 'low health'

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Accepted 24 May 2007

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status mouse houses, which had to operate in parallel for 26 months, are now closed.

During this exercise, the key decisions were to conduct the rederivations in an adjacent but separate self-contained quarantine suite in which mice were housed in isolators and to institute a suitable sampling and microbiological testing programme for isolators to control the quality of the mice. To date, 30 months of follow-up sentinel screening in the MLC indicates that we have achieved our goal of eradicating the endemic infectious agents in our pre-existing, largely conventional colonies. The impact of this rederivation exercise on health and welfare was assessed by comparing the occurrence of specific disease entities in pre-existing conventional colonies with the SPF colonies within the MLC.

Other units upgrading their animal facilities will develop their own programme to improve health status depending on their specific infection problems, access to rederivation technology, caging, colony size, budgets, staffing and building design. This paper aims to highlight some of the broader issues such as the limits of rederivation to sanitize stocks as well as the constraints on health screening in isolators to monitor microbiological status and the acquisition of adequate data to assess the impact on clinical health and welfare.

Materials and methods

Animals

The humane care and use of mice in the rederivation programme was conducted under the Home Office (HO) project licence PPL 30/1704, the *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis programme under PPL 30/2049 and the breeding of inbred mouse stocks in the MLC under the HO Certificate of Designation 30/4303.

Rederivation of mice

The rederivation of mice began in March 2004 and was conducted by the frozen embryo and sperm archive (FESA) core. The protocols used in this study for embryo freezing/thawing, embryo handling, *in vitro* fertilization (IVF) and superovulation techniques are described on the European Mouse Mutant Archive (EMMA) website: www.emmanet.org. Embryo collection, culture and unilateral and/or bilateral transfer of preimplantation embryos into pseudo-pregnant recipient females were performed using the general protocols described by Nagy *et al.* (2003). However, all surgery was performed under general gaseous anaesthesia. Anaesthesia was induced with 4% isoflurane (Abbott Laboratories, Queensborough, UK) then maintained with 1.5% isoflurane throughout the procedure. After surgery, 1 mg/kg body weight of buprenorphine (Vetergesic: Reckitt Benckiser Healthcare, Hull, UK) in 0.1 mL of sterile saline was administered as a subcutaneous injection for postoperative analgesia. The mice were then placed in a warm recovery area before transfer to the appropriate isolator. All mice were observed daily.

All preimplantation embryos were transferred into pseudo-pregnant SPF Crl:CD-1(ICR) female recipients, subsequently referred to as CD-1 females. In the first instance, the CD-1 females were purchased from Charles River, UK and delivered to Harwell on the day of plug. Once CD-1 production was established inside the MLC, the pseudo-pregnant females were generated in-house. Each pseudo-pregnant female was free of the following FELASA listed agents (Nicklas *et al.* 2002): ectromelia, lymphocytic choriomeningitis virus, minute virus of mice (MVM), mouse adenovirus type 1 (FL) (MAV-1), mouse adenovirus type 2 (K87) (MAV-2), mouse cytomegalovirus, mouse coronavirus (mouse hepatitis virus, MHV), mouse parvovirus (MPV), mouse rotavirus (EDIM), pneumonia virus of mice, reovirus 3, Sendai virus, Theiler's murine encephalomyelitis virus (TMEV), *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Helicobacter* spp., *Mycoplasma* spp., *Pasteurella* spp., *salmonella* spp., *Streptobacillus moniliformis*, streptococci β-haemolytic (not group D), *Streptococcus pneumoniae*, endoparasites and ectoparasites. The CD-1 embryo recipients were positive for intestinal protozoan *Entamoeba muris*, but these are considered non-pathogenic commensals.

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Supplemental bacteriological screening data provided by Harlan UK (Harlan UK Ltd Technical Services Department, Loughborough, UK, see below) from routine aerobic culture of the nasopharynx and intestine indicated that the CD-1 mice used in the rederivation programme carried the non-FELASA listed bacteria *Staphylococcus aureus*, *Staphylococcus* spp., α-haemolytic streptococci, *Streptococcus* spp., *Proteus* spp., *Lactobacillus* spp., *Enterobacter* spp. and *Escherichia coli*.

Husbandry

(1) The two pre-existing mouse houses (designated building 371 and building 383, respectively) were operated for an overlapping period of 26 months with the MLC but were finally closed in May 2006. Over 95% of the mouse stocks in these two mouse houses were conventionally housed in open topped cages, the remainder of the mice were housed in IVCs (Charles River, UK). The environmental conditions were 12 h light/dark (07:00–19:00 light phase) cycle, 19–23°C and 45–65% relative humidity. Mice were housed on grade 6 sawdust bedding (Datesand, Manchester, UK). All cages, cage tops, bedding, water bottles and caps were sterilized by autoclaving before use. Water bottles and cages were changed once a week. Husbandry for IVC stocks was performed in a class II biological safety cabinet and these were surface-sterilized with 70% alcohol between different colonies, then wiped down with 2% Trigene spray (Sychem, Chandler's Ford, Hampshire, UK) at the end of the session. Mice were fed RM3 breeding diet (Special Diets Services, Witham, UK) and provided with chlorinated (9–13 ppm) tap water *ad libitum*. All mice were checked daily.

(2) The MLC's quarantine suite is a separate self-contained barrier facility adjacent to the main centre with its own high-efficiency particulate air (HEPA)-filtered system and cage wash facilities. The quarantine suite itself comprises changing rooms, three animal rooms, a stores area, office, staff room, vaporized hydrogen peroxide (VHP) lobby, small general purpose laboratory, a laminar air flow (LAF) cabin connecting the VHP lobby and the small general purpose laboratory and a clean rederivation laboratory. All consumables were brought into the barrier after external sterilization with VHP.

Staff members entering the quarantine suite take a wet-shower before changing into unit clothing and mob cap, before leaving the changing rooms. They then put on over-suits before entering the interior of the facility. All staff leaving the quarantine suite remove their over-suits before entering the changing rooms and then remove their unit clothing before crossing the step over barrier and putting on their outside clothing.

Clean CD-1 recipient females entered the quarantine suite in their home cages through the VHP lobby. The home cages were placed on the LAF cabins platform and the mice transferred to a sterile IVC on the 'clean' side of the LAF cabin. The IVC was then taken to the rederivation laboratory where the embryos were implanted under general anaesthesia on the open bench. After surgery, the mice were allowed to recover inside a sterile IVC before being transferred to the appropriate isolator.

When low-health status mice entered the facility, the home cage was triple-bagged inside the LAF cabin before it was taken straight to the designated isolator.

The quarantine suite supported 20 flexible film isolators (Harlan Isotec, Bicester, UK), 10 of which could accommodate 50 mouse cages. The remaining isolators held 20 mouse cages. Each isolator had its own filtered water supply and stores port. The outer packaging of all supplies entering/leaving the stores port was saturated with 2% Trigene spray for 60 min. The stores port was also used to facilitate the movement of live mice into and out of the isolators. Before entering/leaving the isolator, live mice were placed inside a sterile IVC in the stores port which

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was then saturated with 2% Trigene spray for 10 min. All movements into and out of the isolators were logged on daily record sheets.

Before use, each isolator was fumigated with VHP and tested for sterility before any mice were introduced. An isolator was considered sterile if no bacterial growth was detected in swabs taken from 10 separate locations and cultured in Luria-Bertani (LB) broth (Gibco, Paisley, UK) for three days at 37°C. Isolators containing 'clean' rederived mice were maintained under positive pressure (50–100 Pa of water). However, those isolators that housed mice with a low-health status were maintained under negative pressure (50–100 Pa of water). Both positive and negative pressure isolators were run in parallel from June 2005 onwards.

The environmental conditions in the quarantine suite were: 12 h light/dark (07:00–19:00 light phase) cycle, 19–23°C and 45–65% relative humidity. All consumable items used in the isolators were bagged and autoclaved or irradiated before use. The mice were supplied with chlorinated (9–13 ppm) reverse osmosis water and the contents of the drinking bottles were completely changed once a week after which tests showed chlorine levels >5 ppm. Irradiated RM3 breeding diet was supplied by Special Diets Services, Witham, UK. Food and water was provided *ad libitum*. All husbandry procedures were performed within the isolators and cage bedding was changed weekly. During cage changing, mice were placed in a holding container and the soiled bedding from their cage was scraped out and replaced after a sanitary wipe of the interior bottom of the cage with 2% Trigene. Technicians did not wipe their gloves or disinfect the scraper between cages to promote environmental mixing and cross-contamination with any potential infectious material. Records kept for each rederivation included pregnancy rates, number and gender of pups born, number of pups found dead (removed at daily cage checks), those missing at weaning and those weaned.

(3) The MLC itself is a mouse-only facility, which has been open since June 2004. It has its own HEPA-filtered air system and mice are housed in IVCs (Techniplast UK Ltd). Cages are changed weekly in class II biological safety cabinets, otherwise the food, water and environment are as described for the quarantine suite. On entering, the MLC staff replace their outside clothing with unit clothing, mob cap and over-suit. The staff then pass through an air-shower before entering the main facility. The production ward and wards 2–6 are managed as separate barriers (production staff wet-shower in and change their over-suits before entering the ward).

Sentinel screening

Depending on their availability, C57BL/6J, CBA/CaH, BALB/cAnNCrl, FVB/NCrlBr or CD-1 mice reared in IVCs were used as sentinels.

(1) The pre-existing conventional mouse rooms contained 200–300 cages and one sentinel cage was set up in each room containing four male or female weanling mice; pathogen challenge was by airborne exposure. One sentinel mouse (each exposed for 5–21 weeks) was sent for microbiological testing (see below) from each cage at three-month intervals and tested according to FELASA guidelines (Nicklas *et al.* 2002). For IVC monitoring, sentinel cages were challenged with a 12.5 mL scoop of dirty bedding collected from each of eight cages every week. This screens the 56-cage rack in a seven-week rotation.

(2) Screening the isolators in the quarantine suite comprised two separate groups of CD-1 embryo recipient dams randomly sampled from different boxes and screened before transferring their offspring into the barrier facility. The first four dams were screened when their pups were weaned, and the second four

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were screened three weeks later (6 and 9 weeks post-embryo transfer, respectively) for the full annual FELASA panel. The rationale for having a six and a nine-week screen was to identify and eradicate any infection outbreaks as soon as possible to minimize the possibility of accidental cross-infection between isolators. After two clear rounds of health screening, offspring were transferred to the MLC in sterile IVCs.

(3) The health-screening programme for IVCs in the MLC used either CBA/CaH or CD-1 mice as sentinels and these were challenged by transfer of dirty bedding with one sentinel cage set up per 56 IVC cages, i.e. one side of a cage rack as previously described (Cheeseman *et al.* 2007).

Microbiological testing

Live mice were submitted to an outside laboratory (Harlan, UK) for microbiological testing based on FELASA recommendations (Nicklas *et al.* 2002). The full 'annual' panel was performed twice on mice from the isolators in the quarantine suite. Screening followed the quarterly FELASA programme in the mouse wards within the MLC, the exception being the production ward where inbred strains were screened monthly. The efficient flow of rederivation work through the quarantine facility depended on the timely delivery of microbiological test results. We therefore obtained a service level agreement with Harlan for our results to be provided within seven working days and any positive test results within 1–2 days.

Pathology screening

A total of 1670 mouse necropsies were performed between March 2003 and January 2007. This period spans the rederivation programme and the establishment of SPF colonies in the new SPF facility. The animal care technicians selected cases for examination on the basis of health concerns, whereas phenotyping necropsies were performed for scientific purposes to characterize mouse mutant phenotypes. The number of mice submitted from each stock, their age, gender, the underlying size of the colonies and the ages of the mice at the endpoint of experiments varied considerably. This confounds analysis of the effect of rederivation on disease incidence. To simplify the comparison, we analysed the husbandry and pathology records for a few well-defined clinical disease entities in particular mouse stocks. These disease entities were lactation ileus in C3H/HeH female mice, inflammatory bowel disease (IBD) in transgenic Huntington mice and weight loss in ENU-treated male BALB/cAnNCrl mice. Previously published data were also available for the otitis media (OM) mutant C3H.C-Evi^{Jbo}/H, subsequently referred to as Junbo, in conventional and SPF conditions (Parkinson *et al.* 2006). In addition, we examined the MLC pathology records for the following conditions: abscesses, pyelonephritis and botryomycosis. Mice for necropsy were humanely euthanized by an overdose of barbiturate administered intraperitoneally (Pentoject, Animalcare, York, Yorkshire, UK) and blood for serum was collected by cardiac puncture. Mice were weighed then necropsied and selected organs examined by histology as previously described (Cheeseman *et al.* 2007).

Importation of 'dirty' stocks into quarantine

The rederivation team also import low-health status mice for cryopreservation as part of their remit as a member of EMMA (www.emmanet.org). To improve infection control, mice from colonies with documented health problems were received into a separate isolation facility outside the quarantine suite. Where possible, only dissected male or female reproductive tracts were taken into the quarantine suite prior to harvesting embryos or germplasm. However, when it was essential to expand 'dirty' stocks this was done in the quarantine suite in designated negative pressure isolators. Embryo recipients from these rederivations were housed in a second isolator. If pinworms were endemic in the originating colony, we routinely requested that the mice were treated before shipping. During their stay in quarantine, mice were treated by topical application of ivermectin once a week

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for the first three weeks, then once per month for the next three months. Mice were transferred to a clean cage for treatment and their coats were lightly sprayed with a 10% solution of Panomec (Merial Animal Health, Harlow, Essex, UK) with the intention that the drug was ingested during grooming. The bedding in the home cage was also misted at the same intervals. The treatment was directed by the named veterinary surgeon. In addition to routine CD-1 sentinel screening, postmortem serum samples from imported stocks were tested for MPV, MVM and *Mycoplasma* spp. Currently, sentinels from quarantine isolators containing imported live mice were sampled at six and nine weeks in-line with the rederivation isolators to gather additional information on the rates of transmission of infectious agents within isolators.

Results

Microbiological status of mice before rederivation

Results for a sentinel screen operated from September 2002 to May 2005 are presented in Table 1. In addition to these agents, pathology screens identified single cases of *Mycoplasma pulmonis* lung infection (confirmed by follow-up serology) and *Pneumocystis carinii* in 2003. Cases of *C. piliforme* (Tyzzer's disease) were identified in 1993. Microbiology screens in IVCs, in the pre-existing mouse houses detected pinworms in building 383 IVCs and MHV, were occasionally detected in sentinels and in experimental animals housed in building 371 IVCs (data not shown).

Many of the oocytes, spermatozoa and embryos used for the rederivation exercise were collected from mice from the infected MRC Harwell colonies. This was illustrated in some inbred strains that were bred and weaned in IVCs and were then moved into conventional cages prior to superovulation and oocyte harvesting. In one such example, a group of 19 clinically well, but immunologically naïve, C3H/HeH females, were examined by postmortem histology after oocyte collection and 68% had mucosal MHV syncitial cells in their intestines. MHV syncitia were also found at postmortem in 20% of conventionally-reared, superovulated C57BL/6J females ($n = 10$).

Rederivation

To date, a total of 28,667 embryos have been transferred into 1872 recipient females as part of the ongoing rederivation exercise and an overall littering rate of 27.8% has been achieved. A total of 14,144 of these embryos were generated using IVF and they were transferred into 849 recipient females (28.2% littering rate). The remaining 14,523 embryos were derived *in vivo* from super-ovulated mice and were transferred into 1023 recipient females (26.9% littering rate).

These embryos have been used to resurrect 306 stocks, 127 of which were resurrected using IVF-derived embryos. Hysterectomy rederivation was used to rederive four stocks into the MLC. Live mice from three SPF stocks from commercial suppliers were imported into the MLC without prior rederivation, but only after they had been housed in an isolator and two batches of sentinel mice had been sent off for screening at six and nine weeks post-arrival.

Microbiological screens on rederived and imported mice in quarantine

In 95 isolator cycles, there were two MHV outbreaks that were eradicated by culling all mice and re-sterilization of the affected isolator (Table 1). The first outbreak occurred in an isolator used to rederive six embryo stocks. In this case, 4/4 dams out of 39 recipient females and their 91 pups were positive for MHV antibodies six weeks after embryo transfer (at the time their pups were weaned). Immediate follow-up serology tests confirmed an additional group of five dams from the same isolator were all MHV-positive. At first, suspicion fell on frozen/thawed IVF-derived embryos used to recover two of the six embryo stocks, the other four stocks being derived from frozen/thawed *in vivo* derived embryos. The second MHV outbreak (4/4 positive dams at the 6-week screen) occurred in an isolator used to

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Table 1 Sentinel testing for FELASA agents in conventional mice in the old mouse houses, quarantine isolators and the Mary Lyon Centre (MLC)

		Conventional houses*			MLC‡	
	Test frequency	383	371	Quarantine†	Production	Wards 2–6
Ectromelia	12	0/26	0/23	0/752	0/254	0/143
Lymphocytic choriomeningitis virus	12	0/26	0/23	0/752	0/254	0/143
Minute virus of mice	3	0/108	0/220	0/752	0/316	0/644
Mouse adenovirus type 1	12	0/26	0/220	0/752	0/254	0/644
Mouse adenovirus type 2	3	0/108	13/220 (6%)	0/752	0/301	0/644
Mouse cytomegalovirus	12	0/26	0/23	0/752	0/254	0/143
Mouse hepatitis virus	3	76/108 (70%)	162/220 (74%)	13/757 (2%)§	0/316	0/644
Mouse parvovirus	3	0/108	0/220	0/752	0/316	0/644
Mouse rotavirus	3	0/108	0/220	0/752	0/316	0/644
Pneumonia virus of mice	3	0/108	0/220	0/752	0/316	0/644
Reovirus 3	12	0/26	0/23	0/752	0/254	0/143
Sendai virus	3	0/108	0/220	0/752	0/316	0/644
Theiler's murine encephalomyelitis virus	3	0/108	13/220 (6%)	0/752	0/316	0/644
Citrobacter rodentium	3	0/108	0/220	0/752	0/316	0/644
Clostridium piliforme	3	0/108	0/220	0/752	0/316	0/644
Corynebacterium kutscheri	3	0/108	0/220	0/752	0/316	0/644
Helicobacter spp.	12	4/26 (15%)	2/23 (9%)	8/752 (1%)¶	0/254	0/143
Mycoplasma spp	3	0/108	0/220	0/752	0/316	0/644
Pasteurella spp.	3	61/108 (56%)	101/200 (46%)	0/752	0/316	0/644
Salmonella spp.	3	0/108	0/220	0/752	0/316	0/644
Streptobacillus moniliformis	12	0/26	0/23	0/752	0/254	0/143
Streptococci β-haemolytic not group D	3	0/108	0/220	0/752	0/316	0/644
Streptococus pneumoniae	3	0/108	0/220	0/752	0/254	0/644
Endoparasites					0/316	0/644
Pinworms	3	9/108 (8%)	70/220 (32%)	0/752	0/316	0/644
Tritrichomonas	3	28/108 (26%)	23/220 (10%)	0/752	0/316	0/644
Spironucleus	3	0/108	9/220 (4%)	0/752	0/316	0/644
Entamoeba#	3	0/108	0/220	107/752 (14%)	6/316 (2%)	5/644 (1%)
Ectoparasites					0/316	0/644
Myobia	3	0/108	2/220 (1%)	0/752		

Notes: Depending on the agent. FELASA test frequency is quarterly or annually, the quarantine suite isolators were screened twice for the full panel of agents. MLC screening followed quarterly FELASA programme except in the production ward where inbred strains were screened monthly, see text for details

*Conventional house screening September 2002–May 2005

†Quarantine screening March 2004–January 2007

‡MLC screening June 2004–January 2007

§2 of 95 isolator cycles in the quarantine suite had mouse hepatitis virus (MHV) infections. MHV was detected at six weeks in a total of 13/13 mice tested

¶*Helicobacter* spp. was detected in 2 of 9 low health status quarantine isolators when sentinels were housed with imported stock. This infection has not been detected in rederived mice, see text for details

#*Entamoeba* is considered as a non-pathogenic commensal present in the MLC. *Entamoeba* was endemic in CD-1 female embryo recipients used in the rederivation programme. See text for details

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rederive three of these frozen embryo stocks in 15 recipient females. One of these frozen embryo stocks was common to the first MHV outbreak. We suspected, but could not be certain, that MHV infection was transmitted from these frozen thawed *in vivo* derived embryos, perhaps because of adherent infected material (Peters *et al.* 2006). New batches of embryos were therefore generated for these two stocks and the lines were subsequently rederived free of infectious agents. There was no wave of overt clinical sickness in either MHV outbreak or preweaning mortality. We have also seen transmission of *Helicobacter* spp. (to 100% of sentinels by 6 weeks) from 'dirty' mice to isolator sentinels.

Clinical health and welfare in rederived SPF mice

(1) *Lactation ileus in C3H/HeH female mice*
Lactation ileus (Kunstýř 1986) was a major problem in conventional C3H/HeH mouse colonies because sufficient numbers of foster mothers could not be found for orphaned pups. Typically, there was a peak of sudden-onset deaths or morbidity at 6–15 days postpartum in females in their first lactation cycle (data not shown) with no premonitory signs of illness. Postmortem examination findings were distended intestines with minimal histological pathology and no evidence of a specific viral or bacterial pathogen. In conventional mouse colonies, the morbidity/mortality rates were 115 out of 936 breeding females in one six-month period in 2003–2004. In 30 months operation of the MLC, there have been no cases of lactation ileus in C3H/HeH breeding females. There are currently approximately 300 C3H/HeH females in mating and they have 2–5 litters each. There has been no evidence of postpartum morbidity or mortality associated with the first or second pregnancies.

(2) *Inflammatory bowel disease in transgenic Huntington mice*
In 28 months, 94 transgenic Huntington mice aged between 4 and 20 weeks were culled because of welfare concerns and weight loss and 79% had histological evidence of ulcerative colitis/typhlocolitis, but had no evidence of a specific viral or bacterial pathogen. In the MLC, there have been 18 necropsies performed on Huntington mice. Of these, seven had no clinical signs of illness and the necropsies were performed for scientific interest. One mouse aged 25 weeks had rectal haemorrhage associated with typhlocolitis; and in two further mice aged 49 weeks without clinical signs, one had histological enteropathy and the other had low-grade typhlocolitis.

(3) *Weight loss in ENU-treated male BALB/cAnNCrl mice*
SPF BALB/cAnNCrl males were used in the ENU mutagenesis programme in the pre-existing mouse facility. Although they were housed in IVCs, the barrier sometimes failed and in one batch of 50 mice treated with three 90 mg/kg doses of ENU, half were culled for acute post-treatment and 20% weight loss. Selected males were examined at postmortem and had intercurrent enteropathic MHV infection. Analysis of the health records of the first 24 batches of ENU mutagenized BALB/cAnNCrl and C57BL/6J males ($n = 401$) in the MLC indicated that only a single mouse was culled with weight loss in the immediate post-treatment period. This mouse was not examined at necropsy or by histology, but based on the sentinel screening in the MLC FELASA agents were not contributory.

(4) *Pyogenic infections in SPF mice*
There were 40 cases of pyelonephritis, 14 cases of abscesses (in preputial gland, lung, lymph node and skin) and seven cases of staphylococcal botryomycosis over 30 months in the SPF mouse colonies in the MLC. These tended to be single cases of infection in both males and females in a range of inbred and genetically altered strains of mice. This sporadic pattern of cases in SPF mice was similar to the pattern in conventional mice (data not shown).

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Costs of rederivation

This large-scale rederivation programme for the conventional mouse colonies was built on the expertise of a seven-member FESA team that was already well established in the Mammalian Genetics Unit (MGU) at MRC Harwell. The cryopreservation of MGU stocks was conducted in earnest over a 2.75-year period and represents 5.5 person-years of work. The rederivation programme conducted in MLC quarantine was approximately 3.75 person-years work. The purchase and delivery of pseudo-pregnant CD-1 females cost £25K and additional screening of mice in quarantine cost were £81K alongside the £75K screening costs for the MLC mouse colonies over the same period. To put this in perspective, the construction and equipment costs for the MLC were £18M with an annual recurrent budget (including staff salaries, consumables, power and utilities) of £5M and a combined FESA and MLC staff of 91.

Discussion

We are cautiously optimistic that the rederivation programme has eliminated previously endemic FELASA listed agents MHV, MAV-2, TMEV, intestinal protozoa, pinworms, ectoparasites, *Pasteurella pneumotropica and Helicobacter* spp. The key decision for our success was to perform the programme in a separate quarantine facility equipped with isolators so that infection outbreaks could be contained and decontaminated safely.

The rederivation exercise required the recovery of a variety of genetically altered and inbred strains reflecting the ongoing scientific effort at the MRC Harwell. To facilitate the cryopreservation of many of these strains, we archived two-cell embryos generated by IVF. Our average littering rate of 28%, using embryos harvested predominantly from genetically altered strains, is comparable with published data on the recovery of live pups from frozen/thawed two-cell embryos from inbred mouse strains (Byers *et al.* 2007).

Rederivation of mice, whether by transferring *in vivo*-derived or IVF-derived embryos or by cross-fostering hysterectomy-derived pups, appears to be intrinsically very safe (Reetz *et al.* 1988, Suzuki *et al.* 1996), although it has not been conclusively demonstrated to remove all pathogenic agents that infect mice, e.g. *Mycoplasma* spp. (Hill & Stalley 1991, Homberger & Thomann 1994). Nevertheless, the low risks of disease transmission during the rederivation of mice are multiplied when large number of procedures are performed.

Our isolator screening programme met the FELASA recommendations to screen 3–5 sentinels at increased frequency (i.e. in less than 3-month intervals) (Nicklas *et al.* 2002). The confidence limits for detecting infections using different sample sizes can be determined mathematically based on random sampling of the dams and assumed rates of infection for different agents using the following formula: $n = \log(1 - \text{probability of detecting infection})/\log(1 - \text{assumed infection rates})$ adapted from Lindsey *et al.* (1991). In our screens (a total of $n = 8$ by 9 weeks), there was a 99% probability of detecting a 44% infection rate in at least one animal and a 95% probability of detecting a 31% infection rate. In practice, there is a limit to detecting low rates of infection because the number of mice required for screening is inversely proportional to the percentage of uninfected mice (Nicklas *et al.* 2002). For example, doubling sampling size (and cost) to $n = 16$ would detect an assumed infection rate of 17%, $P = 95\%$ (c.f. $n = 8$ used in this programme to detect 31% infection rate, $P = 95\%$), and sampling aimed at detecting even lower rates of infection would become impractical and prohibitively expensive. A practical approach was to promote infection transmission within isolators to an easily detectable infection level, ideally $\geq$40%. Empirical data from this study indicated that this occurred for some agents. MHV infection was observed to spread rapidly ($\leq$6 weeks) and the 100% infection rate was significantly higher than in sentinels in conventional rooms (13/13 positive cases versus 238/328, $P < 0.02$ Fisher's exact test). Additional information on the spread of infections within isolators came from

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housing 'dirty' imported mice. *Helicobacter* spp. transmitted to 100% of sentinels within six weeks. The lack of transmission of pinworms to sentinels may have been due to effective ivermectin prophylaxis. However, we did not test every individual imported mouse from infected colonies and these mice may not have carried infections. The latter may explain why we have not seen *P. pneumotropica*-positive sentinels after they were housed with mice from *P. pneumotropica*-positive colonies. It is also conceivable that we could import 'dirty' mice that have seroconverted to infectious agents, but do not have active infection and are not infectious to other mice. While relatively low sentinel seroconversion rates for some agents in large conventional rooms (<10% for MAV-2 and TMEV, Table 1) might not equate to low transmission rates in isolators, we attempted to mitigate the effects of slow infection transmission/post-infection seroconversion by including a nine-week screen. As yet, no additional agents have been identified in the latter screen. Extending the isolator screening period beyond nine weeks would have slowed the overall programme and prolonged the risk of having infected mice on site, while the 'dirty' mouse houses were run alongside the new facility. Furthermore, rederived mice have to be young enough to breed well and space constraints did not allow breeding in quarantine.

We based our decisions on whether or not to import low health status live mice after reviewing health-screening health data supplied from the submitting institution. We regarded importation of live 'dirty' stocks into quarantine as potential cross-infection risk while the rederivation programme was underway. To reduce this risk, we ran isolators with 'dirty' mice at negative pressures and those with rederived mice at positive pressures. Special containment has to be considered for live mouse imports if there is a risk they carry agents such as MPV which are shed for an extended period, long-lived in the environment and to which mice seroconvert relatively slowly. Our policy would be to collect sperm or embryos outside the quarantine barrier and avoid long-term holding in quarantine isolators. We also adopted retrospective testing of imported stock for some troublesome infections such as MPV, MVM and *Mycoplasma* spp. as another precaution to assess ongoing risks. None of these agents has been detected to date.

Unfortunately, it was not straightforward to compare clinical health and welfare of the pre-existing conventional mouse colonies with the SPF mice inside the MLC. Potentially useful indicators such as pre-weaning mortalities were difficult to compare because breeding programmes were altered and we imported new founder stock, e.g. DBA/2J, BALB/cAnNCrl and CD-1 from UK suppliers in order to build breeding stocks up quickly. The analysis of the effect of rederivation on disease incidence mice based on our diagnostic and phenotyping pathology records was hampered by uneven sampling between conventional and SPF colonies. Case submission was determined by recognition of mouse ill health (i.e. without matching genetic backgrounds, age, gender, colony size, etc.) or for research interest. However, some inferences can be drawn using selected examples of well-defined disease entities in specific stocks. Before this discussion, it is important to note the limits of our rederivation programme. The CD-1 stocks purchased for use in the rederivation programme, and thereby all stocks rederived into the MLC, were not germfree and carried a number of bacterial agents. Although these were not FELASA listed agents, some such as *S. aureus* and α-haemolytic streptococci can be a cause of clinical disease in immunocompetent mice as well as immunodeficient mice (Nicklas *et al.* 2002). It was beyond the scope of our rederivation programme to remove these non-FELASA listed bacteria from our colonies *S. aureus* and α-haemolytic streptococci may have been the cause of the pyogenic infections we have seen such as the 40 cases of pyelonephritis, 14 cases of abscesses (in preputial gland, lung, lymph node and skin) and seven cases of staphylococcal botryomycosis (Percy & Barthold 2001). These bacterial infections did not seriously hinder individual scientific programmes

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because they occurred only sporadically in individual strains. On the other hand, rederivation programme has excluded the FELASA listed agents in the new mouse colonies and this change in health status has been accompanied by the elimination or marked reduction in three previously important disease entities in conventional mice: lactation ileus in C3H/HeH mice, early weight loss due to IBD in transgenic Huntington mice and substantial weight loss in ENU-treated BALB/cAnNCrl males for which there was direct histological evidence for intercurrent MHV infection. The precise role for individual FELASA agents is unclear as these clinical entities are likely to be multifactorial diseases with possible environmental component(s). The aetiology of lactation ileus is unknown, but it has been suggested that endogenous clostridial infections and/or metabolic exhaustion may be the underlying causes. No specific bacterial pathogens have been identified (Kunstýř 1986). In our C3H/HeH colonies, the susceptible conventional females were fed the same RM3 diet as the unaffected SPF females, so the dietary background was equivalent. The only difference was that the SPF females in the new facility were fed irradiated diet. It is possible that rederivation has eliminated certain key intestinal pathogen(s) responsible for lactation ileus and perhaps also IBD. For instance, under SPF conditions a number of immunodeficient mouse models do not have IBD, but develop IBD after experimental infection with *Helicobacter* spp. (see Discussion in Fox *et al.* 2000). It remains to be determined whether mucosal immunity or gut physiology is impaired in Huntington mice predisposing them to bacterial IBD. The role of MHV in weight loss in ENU-treated mice is perhaps the clearest because ENU is a stem cell mutagen that affects the haematopoietic stem cells causing acute immune suppression and susceptibility to viral infection (Justice *et al.* 2000).

Working in the MRC MGU, it was of considerable scientific interest to see whether some mutant mouse phenotypes would be lost or altered by rederivation. Among the MRC Harwell programmes, none of the neurobehavioural, diabetes, developmental or neuromuscular mutant phenotypes have been lost. However, two altered phenotypes have been recognized after rederivation into SPF conditions. In the imprinted gene mutant C3H101H-Gnas$^{\mathbf{Oedsml\text{-}mat}}$/H, subsequently referred to as *Oed-Sml* (Cattanach *et al.* 2000), almost all conventional *Oed-Sml* on a (C3H/HeH × 101/H)F_1 background mice died within a few days of birth, with fewer than five individuals out of the several hundred born surviving to weaning. Maternal inheritance of the *Oed-Sml* mutation was maintained by crossing to *Mus musculus castaneus*. In contrast, 21 out of 31 rederived SPF *Oed-Sml* on a (C3H/HeH × 101/H)F_1 background generated so far have reached weaning before they were euthanized. Although the numbers of mice are currently small, pre-weaning mortality in *Oed-Sml* mice on a (C3H/HeH × 101/H)F_1 background appears markedly reduced. This breeding programme offers the potential for the better standardization of genetic background in future experiments (Peters J & Cattanach B, personal communication). Another example of an altered phenotype is in the deaf mutant Junbo. The chronic suppurative OM phenotype was milder and late-onset OM under SPF conditions (Parkinson *et al.* 2006). This observation has led to a new line of enquiry to investigate the role of normal nasopharynx staphylococcal and streptococcal flora in OM and an experiment to rear Junbo mice in germfree conditions is now underway. One additional outcome is that new disease phenotypes can come to prominence in SPF conditions. We have recently described exocrine pancreas hypoplasia causing clinical steatorrhoea and runting in SPF C3HeB/FeJ and 101/H pups. This was probably overlooked in the pre-existing colonies because the clinical signs would be similar to some of the endemic gastrointestinal infections such as MHV (Cheeseman *et al.* 2007).

In round terms, the cost of mouse colony rederivation spread over 30 months was £106K, which approximates to 2% of the annual recurrent costs of the MLC, and in terms of manpower approximately 9.25

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person-years of work from a total FESA and MLC workforce of 91. Viewed in the context of the £18M MRC investment in the facility, the cost of rederivation was relatively modest and from the outset it was considered integral to the design and the mission of the MLC. Any cost–benefit analysis has also to take into account the cost to animal welfare, remedial action and the potential interruption to the scientific programmes if we were to deal with a major disease outbreak.

In summary, to our knowledge, we have completed one of the larger one-off mouse rederivation programmes. We adopted the FELASA recommendations for health monitoring mice to help standardize the SPF microbiological quality of animals. Although rederivation techniques are intrinsically safe, we took the precaution of rederiving mice in isolators in a separate quarantine facility. This facilitated the containment and decontamination of two MHV infection outbreaks emphasizing that the overall success of a rederivation programme depends on coupling high standards of embryo sanitization with effective screening for infectious agents within isolators. It is also clear that the improvement in microbiological status of the mice translates into better health and welfare standards. Reducing the prevalence of specific clinical diseases in strains of mice minimizes the number of mice bred to complete experiments. In addition, the refinement and standardization of microbiological status simplifies experimental design and paves the way for better science. The benefits also extend to improving the efficiency of archiving mouse stocks and upgrading the general health status of the mutant mice which are distributed to the wider scientific community.

Acknowledgements The FESA team rederived the mice. John Bowler organized the sentinel screening programme. Nikki Chrobot, Kate Vowell, Dave Shipston and Jim Humphreys performed necropsies on the mice, and Adele Austin, Caroline Barker, Jennifer Corrigan and Terry Hacker produced histological sections. We thank the staff of the MLC for their care of the mice. Pete Glenister was instrumental in initiating the FESA and planning the quarantine suite in the MLC. We thank Adrian Deeny and those at Harlan UK for diagnostic services.

Addendum

In the interval between writing this paper, and its going to press, a further 29 rederivation isolation cycles have been completed in quarantine without a disease breakdown. The rolling programme of sentinel screens in the MLC wards shows that the colony remains free of FELASA agents.

References

Byers SL, Payson SJ, Taft RA (2007) Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). *Theriogenology* **65**, 1716–26

Cattanach BM, Peters J, Ball S, Rasberry C (2000) Two imprinted gene mutations: three phenotypes. *Human Molecular Genetics* **9**, 2263–73

Cheeseman M, Chrobot N, Fray M, Deeny A (2007) Spontaneous exocrine pancreas hypoplasia in specific pathogen free C3HeB/FeJ and 101/H mouse pups causes steatorrhea and runting. *Comparative Medicine* **57,** 210–16

Fox JG, Dangler CA, Schauer DB (2000) Inflammatory bowel disease in mouse models: role of gastrointestinal microbiota as pro inflammatory modulators. In: *Pathology of Genetically Engineered Mice* (Ward JM, Mahler JF, Maronpot RR, Sundberg JP, Frederickson RM, eds.) Ames, IA: Iowa State Press, 299–315

Hill AC, Stalley GP (1991) *Mycoplasma pulmonis* infection with regard to embryo-freezing and hysterectomy derivation. *Laboratory Animal Science* **41**, 563–6

Homberger FR, Thomann PE (1994) Transmission of murine virus and mycoplasma in laboratory mouse colonies with respect to housing conditions. *Laboratory Animals* **28**, 113–20

Justice MJ, Carpenter DA, Favor J, *et al.* (2000) Effects of ENU dosage on mouse strains. *Mammalian Genome* **11**, 484–8

Kunstýř I (1986) Paresis of peristalsis and ileus lead to death in lactating mice. *Laboratory Animals* **20**, 32–5

Lindsey JR, Boorman GA, Collins MJ, Hsu C-K, Van Hoosier GL Jr, Wagner JE (Committee on Infectious Diseases of Mice and Rats) (1991) Health Surveillance Programs. In: *Infectious Diseases of Mice and Rats.* Washington DC: Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 21–7

Nagy A, Gertsenstein M, Vintersten K, Behringer R (2003) *Manipulating the Mouse Embryo: A Laboratory Manual*. 3rd edn. New York: Cold Spring Harbor Laboratory Press

Nicklas W, Baneux P, Boot R, *et al.* (2002) Recommendations for health monitoring of rodent and rabbit colonies in breeding and experimental units. *Laboratory Animals* **36**, 20–42

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Parkinson N, Hardisty-Hughes RE, Tateossian H, *et al.* (2006) Mutation at the *Evi1* locus in *Junbo* mice causes susceptibility to otitis media. *PLoS Genetics* e149

Percy DH, Barthold SW (2001) Infections with Gram-positive bacteria. In: *Pathology of Laboratory Rodents and Rabbits*. 2nd edn. Ames, IA: Iowa State University Press, 62–6

Peters DD, Marschall S, Mahabir E, *et al.* (2006) Risk assessment of mouse hepatitis virus infection via *in vitro* fertilization and embryo transfer by use zona-intact and laser-microdissected oocytes. *Biology of Reproduction* **74**, 246–52

Reetz IC, Wullenweber-Schmidt M, Kraft V, Hedrich HJ (1988) Rederivation of inbred strain of mice by means of embryo transfer. *Laboratory Animal Science* **38**, 696–701

Suzuki H, Yorozu K, Watanabe T, Nakura M, Adachi J (1996) Rederivation by means of *in vitro* fertilization and embryo transfer. *Experimental Animals* **45**, 33–8

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